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(54) Title: COMPOSITIONS OF TOCOPHEROL AND BETA-CAROTENE

(57) Abstract

A method for the prevention or treatment of atherosclerosis in a mammal, said method comprising administering an effective amount of natural tocopherol and natural carotene to prevent or treat atherosclerosis in a mammal in need of such prevention or treatment. A pharmaceutical composition for the prevention or treatment of atherosclerosis, which comprises an effective unit dosage amount of natural tocopherol and natural carotene to prevent or treat atherosclerosis and a pharmaceutically acceptable carrier therefor.

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COMPOSITIONS OF TOCOPHEROL AND BETA-CAROTENE

Field of the Invention

The present invention relates to pharmaceutical compositions which exhibit a protective effect against the development of atherosclerosis. These compositions comprise the combination of natural tocopherol and natural beta-carotene in a pharmaceutically acceptable carrier. The present invention also includes within its scope methods of preventing the development of atherosclerosis and the resulting cardiovascular disease, e.g., coronary artery disease by administering an effective amount of the composition of the present invention to prevent atherosclerosis.

Background

It is generally recognized that many factors contribute to the development of cardiovascular disease. These factors include, for example, smoking, obesity, hypertension, hyperlipidemia and hypercholesterolemia. Hyperlipidemia and hypercholesterolemia contribute to the accumulation of fatty substances on the arterial wall (atherosclerosis) resulting in narrowing of coronary blood vessels and the development of ischemic heart disease.

In the articles "Vitamin E Consumption And The Risk Of Coronary Disease In Women" by Stampfer et al., The New England Journal of Medicine, 328: 1444-9 (1993), and "Vitamin E Consumption And The Risk Of Coronary Heart Disease In Men" by Rimm et al., The New England Journal of Medicine, 328: 1450-6 (1993), it was disclosed that oxidation of low-density lipoprotein (LDL) plays a role in atherosclerosis. Thus, the oxidation of LDL increases their incorporation into the arterial intima which is an essential step in atherogenesis.

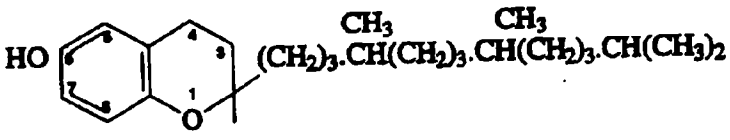
Consequently, a variety of dietary and drug regimen have been developed or proposed which would block the oxidative modification of LDL. These regimen usually include the ingestion of vitamin E alone.

Thus, in the two articles, cited above, the investigators studied the effect of administering vitamin E and the risk of coronary disease and observed that the use

of vitamin E supplements in middle-aged women is associated with a reduced risk of coronary heart disease. Similarly, an association between a high intake of vitamin E and a lower risk of coronary heart disease was also observed in men.

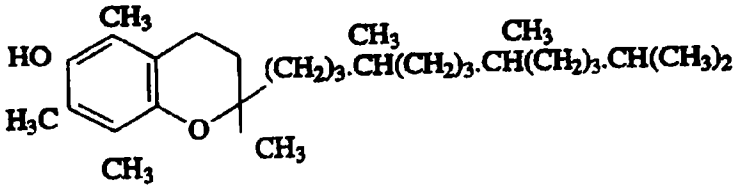
In another study reported in Lancet, 342:1379-84 (1993), it was observed that high beta-carotene intake reduce the risk of myocardial infarction. Beta-carotene has also been suggested as useful in reducing vascular events in patients with chronic stable angina. See, Gaziano et al., "Beta Carotene Therapy for Chronic Stable Angina", Circulation, 82:III, Abstract No. 0796 (1990).

Tocopherols which occur in nature as alpha-tocopherol, beta-tocopherol, gamma-tocopherol and delta-tocopherol possess vitamin E activity. Chemically, they may be considered as having a "tocol" nucleus of the formula I:

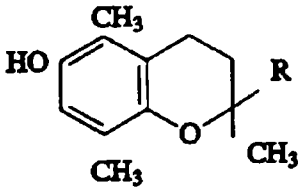


I

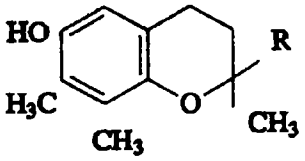
The naturally occurring alpha, beta, gamma, delta- tocopherols, respectively, differing from one another in the number or position of the methyl groups on the chroman ring of (I) have the following structural formulas II, III, IV, and V:



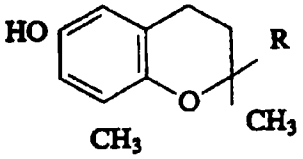
II



III



IV



V

wherein R is $(CH_2)_3-CH(CH_3)(CH_2)_3CH(CH_3)(CH_2)_3CH(CH_3)_2$

These compounds possess antioxidative activity due to the presence of the phenolic hydroxy group at C-6 of the above compounds II, III, IV and V. These

hydroxy groups are extremely susceptible to oxidation. Thus, they protect less susceptible compounds by breaking up the chain of oxidation reactions.

The presence of these phenolic hydroxy groups permit the formation of esters. Both the free tocopherols and their esters are insoluble in water and soluble in fats and oil. Hence, they are also known as "fat-soluble vitamins."

While the precise role of vitamin E in human nutrition has not been clearly established, it has been suggested, that its deficiency may cause a variety of manifestations such as sterility, myocardial degeneration and necrosis of the liver. The daily requirement is suggested to be about 30mg per day for a 70 kg adult human.

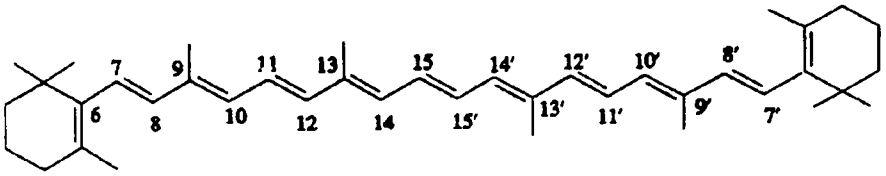
Carotenoids also occur in nature, mainly in plants. They are largely responsible for the yellow to red colors of many plants, particularly edible vegetables, such as carrots and squash. Chemically they are unsaturated, polyisoprene hydrocarbons. Over 500 carotenoids are known, e.g., alpha, beta, and gamma carotene. The most widely used is beta-carotene because it is a precursor of vitamin A. The structural formula is described below (see VI). The daily requirement of vitamin A as suggested by National Research Council for maintenance of good health is 1,000 Retinol equivalents (RE) for males and 800 RE for females. (1,000 RE is equivalent to 5,000 International Unit.) The relation between carotene and vitamin A is 6 to 3.44 by weight of the respective pure compounds.

For a more detailed description of tocopherol and beta-carotene, see Bailey's Industrial Oil and Fat Products, pp. 69-98 (John Wiley & Sons, Inc., New York, New York, 1979).

Brief Description of the Prior Art

Tocopherols and vitamin A have been formulated in compositions with other vitamins as dietary supplements or with other compounds to control cholesterol. U.S. Patent 4,034,983 discloses compositions of polyol fatty acid polyesters such as sucrose octaoleate with vitamins, e.g., vitamin E. U.S. Patent 4,005,196

discloses similar types of formulations, i.e., sucrose octacleate, with vitamins. U.S. Patent 5,278,189 describes compositions of ascorbate with liprotein binders which include tocopherol and beta-carotene having the following structural formula:



VI

Beta-carotene is a precursor of vitamin A. Thus, it is metabolized in the body to form vitamin A by cleavage of the double bond at the 15,15' position of compound VI.

The role of vitamin A in human nutrition is known. For example, vitamin A deficiency leads to night blindness. It also plays an essential role in the growth and the formation of epithelial tissues. Like the tocopherols, beta-carotene acts as antioxidant in the formulation.

In a paper "Increased Preferential Absorption in Human Atherosclerotic Plaque with Oral Beta Carotene," Circulation, 1988, 78:338-344, it was reported that oral beta-carotene may influence selective ablation of atherosclerotic plaques.

While the art recognizes the role of vitamin E in atherosclerosis as described in the aforementioned articles in The New England Journal of Medicine, and synthetic beta-carotene is available as "Solatene" from Hoffmann-LaRoche, Nutley, N.J., the prior art does not teach the protective or enhancing effect of natural tocopherols (vitamin E) on the cardiovascular system by combining the natural tocopherols with a particular blend of natural carotenes as described below. Such a combination is particularly beneficial in protecting the blood vessels from

developing atherosclerosis and hence the prevention of cardiovascular disease.

Summary of the Invention

Accordingly, a primary object of the present invention is to provide compositions which exert a protective action against atherosclerosis and hence the prevention of the development of cardiovascular disease.

Broadly speaking, these compositions comprise the combination of an effective antioxidant amount of natural tocopherol and a blend of natural carotene in a pharmaceutically acceptable carrier suitable for oral or parenteral administration. These compositions in which the protective effect of natural vitamin E has been enhanced by natural carotene blocks the oxidation of low-density lipoproteins in serum of mammals, including humans.

Detailed Description of the
Preferred Embodiments of the Invention

According to the present invention tocopherols which primarily comprise a mixture of compounds II, III and IV in the compositions are present in an effective antioxidant amount. Typically these compounds are present from about 50 to 1000 International unit per dosage unit.

Natural tocotrienols and natural tocopherols are derived from vegetable oils. Soy oil is the most widely used source. Sunflower, corn, peanut, rapeseed and cottonseed oils may also be used. Natural tocotrienol and natural tocopherols are very different from that produced by chemical synthesis, i.e., synthetic "vitamin E." While the definition of vitamin E is not consistent, for the purposes of the present invention, vitamin E refers to both tocotrienols and tocopherols.

Synthetic vitamin E is a mixture of eight different stereoisomers, only one of which is molecularly equivalent to natural vitamin E. The other seven stereoisomers have a lower biological activity. The mammalian body prefers the natural stereoisomer.

Natural vitamin E is recognized as having 36 percent greater potency than

synthetic vitamin E. Recent studies suggest that natural vitamin E is probably twice as effective as synthetic vitamin E.

Natural vitamin E also remains in the body much longer than synthetic vitamin E. The seven synthetic stereoisomers are secreted into the bile and then into the intestine for removal from the body. The natural vitamin E stereoisomer, on the other hand, is returned to the bloodstream in the form of low density lipoproteins.

Any natural tocopherol or tocotrienol, its ester or compounds convertible to either tocopherols or their esters are suitable for use in the practice of the present invention.

The prior art has failed to appreciate any benefit associated with the administration of tocotrienols and tocopherols to a mammal, including humans, to prevent the harmful effects of atherosclerosis. Further, the prior art has heretofore never recognized any benefit for such a method using natural tocotrienols and natural tocopherols.

With respect to the natural carotene component of the present invention, the natural carotene blend which will be described below is particularly preferred and is typically present from 5 to 50 mg per dosage unit.

The preferred naturally occurring carotene blend particularly suitable for the present invention has the following approximate composition:

Beta-carotene	85-90%	(approximately a 1:1 mixture of cis-isomers and trans-isomers)
Alpha-carotene	10-5%	
Lutein	2.5%	
Zeaxanthin	2.5%	

Other natural beta-carotene blends, such as those isolated from palm oil, containing typically about 65% all trans beta-carotene and 35% alpha-carotene may be used in the present invention as well.

Lutein and zeaxanthin are naturally occurring substances from vegetable

sources. Natural lycopene is found, for instance, in tomatoes. The natural enhance the biological effects of natural beta-carotene. For a fuller description of each of the foregoing compounds, please see Dictionary of Organic Compounds, Vol. 5.

Beta-carotene, provitamin A, is readily metabolized by the body into vitamin A when required. There is also increasing evidence which suggests that there is a therapeutic benefit to beta-carotene itself, independent of vitamin A activity.

The primary source of the beta-carotene is an algae named *Dunaliella salina*. The algal cell functions just like an ordinary plant cell. It is photosynthetic, converting carbon dioxide from the atmosphere into cell material and to provide energy. This is done by the green chlorophyll in the cell which is normally not visible as it is masked by the intense orange color of the beta-carotene.

Natural beta-carotene from the algae comprises an approximately equal mixture of cis and trans isomers with the cis form of beta carotene being more soluble in oil than synthetic trans beta carotene.

Synthetic beta-carotene is derived from synthetic organic chemicals and is a crystalline form of beta-carotene, primarily the trans isomer (a molecular configuration). The synthetic form is not the focus of the present invention which is directed to natural source products because of the advantages associated with their use.

The synthetic crystals of beta-carotene are difficult to dissolve in organic chemical solvents, implying that the human body would have similar, or greater, difficulties in assimilating the compound.

The natural carotenoids are mixture of compounds. Those include beta-carotene, alpha-carotene, lutein, cryptoxanthin, zeaxanthin and lycopene.

The natural carotinoids are a mixture of cis and trans isomers while the synthetic carotenoids are all trans isomers.

Betatene, natural mixed carotenoids, is a registered trademark of Betatene Ltd. and is particularly useful in the practices of the present invention.

Betatene is a deep red suspension of natural mixed carotenoids in vegetable oil. The mixed carotenoids are isolated from the sea algae *Dunaliella salina*.

Betatene, natural beta-carotene, is soluble in oil to about 3.7% or about ten times the solubility of synthetic oil suspensions. This indicates a higher degree of bioavailability in the body.

The carotenoid content of Betatene 20% is standardized to contain not less than 200 mg per gram of five naturally occurring carotenoids that are commonly found in various fruits, cruciferous, yellow, and dark green leafy vegetables. The typical carotenoid distribution in Betatene 20% is as follows:

	200 mg/gram
beta-carotene	190,500 mcg
alpha-carotene	6,000 mcg
zeaxanthin	1,200 mcg
cryptoxanthin	1,400 mcg
lutein	900 mcg

In addition to its role as an antioxidant, the beta-carotene provided by Betatene 20%, is a safe source of vitamin A, being converted to vitamin A within the body only as needed.

The oral compositions can be made by conventional compounding procedures known in the pharmaceutical art, that is, by mixing the active substances with edible pharmaceutically acceptable non-toxic inert, solid or liquid carriers and/or excipients suitable for systemic administration and conventionally used in oral dosage forms. Additionally, edible, non-toxic pharmaceutically acceptable stabilizers usually used as stabilizers in oral dosage forms or edible, non-toxic pharmaceutically acceptable salts thereof as well as ascorbic acid can be included in the compositions. All the above carriers, excipients and stabilizers are intended to include only those suitable for oral administration and all are conventional and known to the pharmaceutical compounding art.

The pharmaceutical compositions for oral administration may be in the form of tablets, including sustained release forms, lozenges, chewing gum, and capsules. The soft gelatin capsule dosage form is most preferred. These dosage forms are prepared by those skilled in the art. Thus, for example, about 500 units of

tocopherol and 25 mg of the beta-carotene blend as described above are blended with a sufficient amount of arachis oil to make about 450 mg. It is then dispensed into a soft gelatin capsule, the capsule is sealed by steam.

What is claimed is:

1. A method for the prevention or treatment of atherosclerosis in a mammal, said method comprising administering an effective amount of natural tocopherol and natural carotene to prevent or treat atherosclerosis to a mammal in need of such prevention or treatment.

2. The method according to claim 1 wherein approximately 50 to approximately 1000 international units of tocopherol and approximately 5 to approximately 50 mg of beta-carotene are administered.

3. The method according to claim 1 wherein said natural tocopherol and natural carotene are orally administered at least once per day.

4. A pharmaceutical composition for the prevention or treatment of atherosclerosis, which comprises an effective unit dosage amount of natural tocopherol and natural carotene to prevent or treat atherosclerosis and a pharmaceutically acceptable carrier therefor.

5. The composition according to claim 4 which comprises 50 to 1000 international units of tocopherol and 5 to 50 mg of beta-carotene per dosage unit.

6. The composition according to claim 4 wherein said carotene has the following composition:

Beta-carotene	85-90%	(approximately a 1:1 mixture of cis-isomers and trans-isomers)
Alpha-carotene	10-5%	
Lutein	2.5%	
Zeaxanthin	2.5%	

7. The composition according to claim 6 in which carotene isolated from palm oil containing about 65% all trans beta-carotene and 35% alpha-carotene.

8. The composition according to claim 6 further comprising lycopene.

9. A method for blocking the oxidation of LDL in serum which comprises contacting said serum with the composition as defined in claim 4.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16769

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/355, 31/07.
US CL :514/458, 725.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/458, 725.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS;
TERMS SEARCHED;CAROTENE AND TOCOPHEROL AND ATHEROSCLEROSIS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	THE LANCET, vol. 342, issued 1993 , KARDINAAL, ET. AL. "ANTIOXIDANTS IN ADIPOSE TISSUE AND RISK OF MYOCARDIAL INFARCTION: THE EURAMIC STUDY", pages 1379-1384. see summary.	1 — 2-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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